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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/20 — A1

(11) International Publication Number: WO 91/02973

(43) International Publication Date: 7 March 1991 (07.03.91)

(21) International Application Number: PCT/US90/04699

(22) International Filing Date: 20 August 1990 (20.08.90)

(30) Priority data:
396,446 — 21 August 1989 (21.08.89) — US

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(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: POROUS POLYMER FILM CALCIUM ION CHEMICAL SENSOR AND METHOD OF USING THE SAME

(57) Abstract

A method of measuring calcium ions is disclosed wherein a calcium sensitive reagent, calcichrome, is immobilized on a porous polymer film. The reaction of the calcium sensitive reagent to the Ca(II) is then measured and concentration determined as a function of the reaction.

* See back of page

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POROUS POLYMER FILM CALCIUM ION CHEMICAL SENSOR AND METHOD OF USING THE SAME

BACKGROUND OF THE INVENTION

Chemical sensors are used to detect the presence of a metal cation in a substance and are useful in a variety of fields. Typically, a colorimetric reagent is immobilized on a support, such as optical fibers, and the sensor is applied in biomedical uses, process control, and environmental analysis. Detection of calcium ions (Ca(II)) is useful, for example, in detecting the amount of calcium in the blood of animals, or in detecting calcium in the food processing industry. The colorimetric reagent reacts with the calcium ion in a substance or solution so that there is a change in an optical property; for example, absorption, luminescence, or reflectance, and correlated with the concentration of the calcium ion. This is accomplished by correlating the concentration of the calcium ion to a calibration curve which relates the optical property to the concentration of the calcium.

Sensors which have been developed to this point have several deficiencies, including a slow response time because of barriers to mass transport at the polymer support, a low sensitivity due to weak analytical signal, a low selectivity due to inter-

ferences, and a long term instability because of degradation of the immobilized reagent or its desorptive loss from the support.

This invention relates to an improved sensor which provides for a rapid response time and high sensitivity through the use of a porous polymer film as the support material for the analytical reagent. High selectivity, and resistance to degradation are achieved by employing a calcium ion sensitive colorimetric reagent having sulfonic acid or carboxylic acid binding to the anion exchange polymer and calcium ion chelating groups.

A primary object of the invention is to provide for an improved chemical sensor of calcium ions in a solution or substance.

It is an objective of this invention to provide a chemical sensor for calcium ions which has a rapid response time.

A further object of the invention is to provide for a highly sensitive chemical sensor of calcium ions.

Yet a further object of the invention is to provide a chemical sensor with high selectivity for calcium ions.

Another object of the invention is to provide a chemical sensor which is resistant to degradation.

A still further object of the invention is to provide a chemical sensor in which the analytical reagent has strong binding to the underlying polymeric support.

Further objects of the invention will become apparent in the following disclosure.

SUMMARY OF THE INVENTION

A porous anion exchange polymer film is provided which has immobilized on it a calcium ion sensitive colorimetric reagent, preferably calcichrome. The reaction of the calcium ion analyte may be measured using the colorimetric reagent.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph with Ca(II) concentration as the x-axis and relative reflectance as the y-axis.

DETAILED DESCRIPTION OF THE INVENTION

The anion exchange polymer film used in this invention is well known to those skilled in the art, however, a critical aspect is that the film must be porous. Enhanced porosity insures rapid response, and the large number of binding sites offered by a polymeric support provides a strong analytical signal. Such a porous anion exchange polymer film is available commercially and can be obtained, for example, from RAI Research Corporation in Hauppauge, New York, under the classification "N0030".

Quaternized amines are used for this embodiment of the polymer film. The positive charge from the quaternized amines allows the calcichrome to stick to the film, and porosity assists in the improved response time. While quaternized amine polymers are preferred with the film, a polymer film which is perm-selective provides the desired properties. Such a film is permeable only to small molecules, and prohibits the permeation of macromolecules.

The degree of porosity will depend upon what is being analyzed. The film should be porous enough to allow the analyte, calcium, to pass through while preventing permeation of contaminants. In using the film for blood serum analysis, for example, the smallest degree of porosity preferred would be about 5,000 angstroms, and the largest 10 microns. The preferred range would be 0.5 microns up to 10 microns, with 10 microns the most preferred.

Immobilized on the film is a Ca(II) sensitive colorimetric reagent. Any agent with sulfonic acid or carboxylic acids that bind to the anion exchange polymer, and also contain Ca(II)-chelating groups provides the satisfactory response of the invention. However, it is necessary that the reagent retain its Ca(II)-chelating properties after being immobilized on the support. The effective reagent discovered in

this invention is calcichrome, also referred to as calcion. Calcichrome is 2,8,8-trihydroxyl-1,1-azonaphthalene-3,6,3,6,tetrasulfonicacid. Calcichrome is applied to the support in a concentration of $5X10^{-4}$mM/g to $2X10^{-2}$mM/g of dry films. The preferred concentration is $1X10^{-2}$mM/g of dry film.

Several methods are available for trapping the reagent on the film and include covalent binding, electrostatic binding, adsorption, or trapping the analyte in a solution reservoir behind a membrane. With electrostatic binding calcichrome negative charges are attracted to the amine positive charges. In adsorption, a strong chemical bond is not employed, but a variety of interactions retain the reagent on the film. The calcichrome can be trapped behind a membrane, such as Teflon or cellulose, so that the calcium can diffuse through and react with the calcichrome. The specific method employed for trapping the reagent is not critical.

If impregnating the calcichrome onto the film, the timing is not critical, and satisfactory results are obtained when a time period from about five minutes to overnight is employed. Optionally, the film may be air-dried for storage or used immediately. When used in the optical sensing of Ca(II), it

is preferable that the sample solution be at or about pH 12.

Once the calcichrome is immobilized on the film, the optical and/or chemical properties of the sensor are then evaluated as the function of the Ca(II). An example of a method of evaluating such properties is through the use of diffuse reflectance spectroscopy. A standard is used to which the results may be compared in order to determine the concentration of the Ca(II).

By using the invention disclosed, it is possible to obtain a reaction time in a much shorter time period than is obtained with other methods. Usually, with present methods, one must wait five to ten minutes in order to get a reaction. Here, the results are obtained in far less than one minute and can be obtained in as short a time as fifteen seconds or less.

The following is presented as a means of illustrating the invention, and is not intended to limit the process. Variations will be evident to one skilled in the art as falling within the scope of the invention.

Example

Calcichrome, obtained as its tetra-sodium salt (Pfalz & Bauer, Inc., Waterbury, Connecticut) was

immobilized at the porous anion exchange polymer film (RAI Research Corporation, Hauppauge, New York) by immersing a film in an aqueous calcichrome solution at room temperature for several hours. After removal from the solution, excess reagent was washed from the substrate with deionized water.

Both optode and flow cell for the chemical sensor were constructed to use in testing the chemical sensor feasibility. Optical fibers (400 m diameter, Ensign-Bickford Optics Co., Avon, Connecticut) were used as light transmission lines in both designs. Incoming light was transmitted by the first array of optical fibers and the diffused reflected light at the sensor film was collected by the second array of optical fibers and transmitted to a monochromator. A Xenon arc lamp (Oriel Corporation, Stanford, Connecticut) was used as a light source; the transmitted radiation was dispersed with a 0.22 m grating monochromator and monitored with a digital photometer (Spex DPC-2, SPEX, Inc., Edison, New Jersey) which was operated in an analog mode.

The optical response of calcichrome immobilized at a porous anion exchange polymer film in the absence or in the presence of Ca(II) of pH 12.1 was then determined. The resulting calibration curve plotting relative reflectance to Ca(II) concentra-

tions is shown in figure 1. This enabled the concentration of Ca(II) to be determined. The limit of detection of the sensor obtained under the stated conditions is in the order of mM. The transient optical response of the sensor was measured by injecting a Ca(II) solution at pH 12.1 through the flow cell. Equilibration (99% completion) was achieved in about 13 seconds; the response reaches 63% (1-1/e) of its maximum reflectance value in about 3.7 seconds.

The stability of the sensor response was examined by measuring the diffuse reflected light intensity before and after prolonged exposure of incident light, and between successive days. No significant difference in intensity was observed.

The interferences of other metal ions were examined using Mg(II), Ba(II) and Sr(II) as the test ions. The optical response was measured in pH 12.1 buffer and in saturated solutions of these ions at pH 12.1. The reflectance spectra obtained were almost indistinguishable.

Thus it can be seen that the invention accomplishes at least all of its objectives.

We claim:

1.

A method of measuring calcium in a solution comprising:

immobilizing a calcium sensitive porous reagent on porous polymer film;

contacting said film with said calcium containing solution;

measuring reaction of said calcium sensitive reagent to said calcium containing solution; and

determining calcium concentration in said solution as a function of said reaction of the calcium sensitive reagent.

2.

The method of claim 1 wherein an agent with an acid selected from the group consisting of sulfonic acid and carboxylic acid capable of binding to said polymer film, and having calcium chelating groups is used as said calcium sensitive reagent.

3.

The method of claim 1 wherein calcichrome is used as said calcium sensitive reagent.

4.

The method of claim 1 wherein quaternized amine is used as said porous polymer film.

5.

The method of claim 3 wherein said calcichrome is concentrated on said film at $5X10^{-4}$mM/g to $2X10^{-2}$mM/g.

6.

The method of claim 1 wherein said calcium sensitive reagent is immobilized on said film by electrostatic binding.

7.

The method of claim 1 wherein said calcium sensitive reagent reaction is measured by diffuse reflectance spectroscopy.

8.

The method of claim 1 wherein said reaction occurs in up to one minute.

9.

The method of claim 1 wherein said reagant is immobilized on a film having a porosity to allow said calcium in said solution to permeate said film while prohibiting permeation of other molecules.

10.

The method of claim 1 wherein said method is used for blood serum analysis and said reagent is immobilized on a film having a porosity of about 5,000 angstroms up to about 10 microns.

11.

A sensor for measuring the concentration of calcium in a solution comprising a porous polymer film having a calcium sensitive reagent immobilized thereon.

12.

The sensor of claim 11 wherein said calcium sensitive reagent is an acid selected from the group consisting of sulfonic acid and carboxylic acid capable of binding to said polymer film and having calcium chelating groups.

13.

The sensor of claim 11 wherein said calcium sensitive reagent is calcichrome.

14.

The method of claim 11 wherein said porous polymer film contains quaternized amine.

15.

The sensor of claim 13 wherein said film contains calcichrome at a concentration of $5X10^{-4}$mM/g to $2X10^{-2}$mM/g.

16.

The sensor of claim 15 wherein said film contains calcichrome at a concentration of $1X10^{-2}$mM/g.

17.

The sensor of claim 11 wherein said film has a porosity to allow said calcium in said solution to permeate said film while prohibiting permeation of other molecules.

18.

The sensor of claim 10 wherein said film has a porosity of about 5,000 angstroms up to about 10 microns.

19.

The sensor of claim 18 wherein said film has a porosity of about 0.5 microns up to about 10 microns.

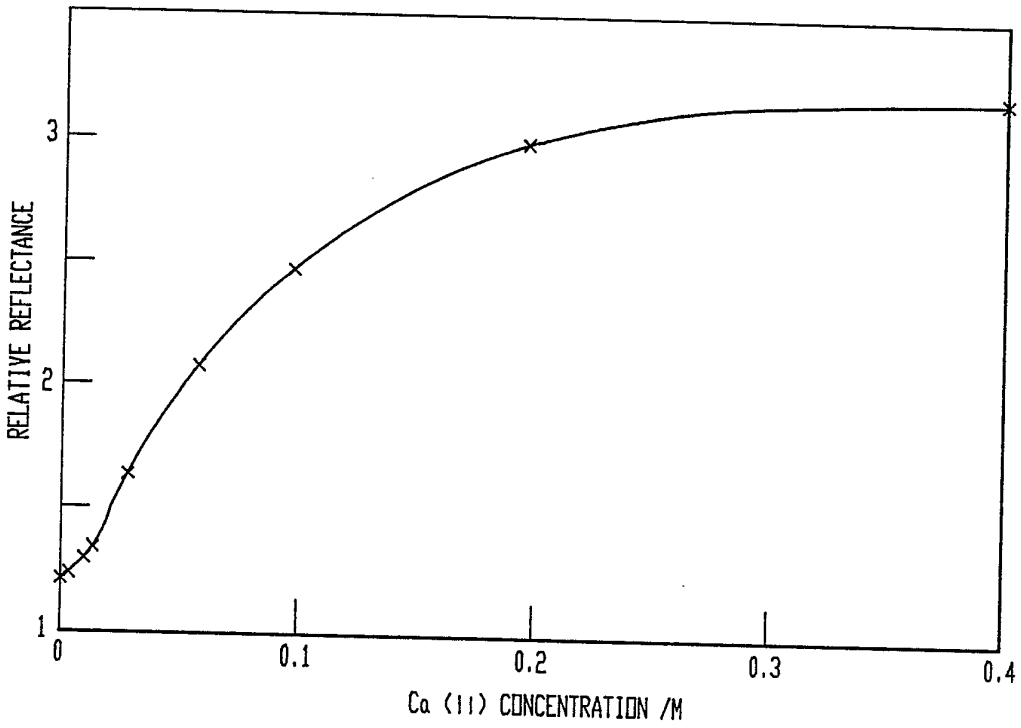


FIG.1

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/04699

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 3

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): G01N 33/20
U.S. Cl.: 436/79

II. FIELDS SEARCHED

Minimum Documentation Searched 4

Classification System 5	Classification Symbols
U.S.	436/74,79 422/55,56

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 6

APS SEARCH (1975-1990) - SEE ATTACHED FOR SEARCH TERMS
CHEMICAL ABSTRACTS DATA BASE

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
X Y	US, A, 4,594,225 (Arai et al.) 10 June 1986. See the entire document.	1,7,11 3,5,6,9 10,13,15-19
Y	Sigma Chemical Company (SaintLouis USA), February 1986, Page 229. See entire document.	3,5,6,9 10,13, 15-19
Y	US, 2,583,890 (Schwarzenbach) 29 January 1952. See the entire document.	2,12
A	US, A, 3,635,679 (Bloch et al.) 18 January 1972. See the entire document.	
A	US, A, 3,754,865 (Gindler) 28 August 1973. See the entire document.	
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IV. CERTIFICATION

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
20 November 1990	29 JAN 1991
International Searching Authority 1	**Signature of Authorized Officer 20**
ISA/US	Jeanne A Massie for David Redding

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE FIRST SHEET
(Not for publication)

DATA BASE SEARCH TERMS

I. File US PAT

. Calcichrome or calcion
. Sensor
. Porous Film
. Quarternized Amine
. Blood
. Sulfonic acid or carboxylic acid
. Electrostatic binding
. Diffuse Reflect spectroscopy